Carcinogen Prediction in the Laboratory: A Personal View [and Discussion]
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Carcinogen prediction in the laboratory: a personal view

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Although carcinogens can be divided into various categories, i.e. viruses, physical agents and synthetic and naturally occurring chemicals, it is the latter that give rise to the greatest concern because of their number, quantity and distribution. Present methods of testing chemicals for potential carcinogenicity rely in the main on administration of these at maximally tolerated dose levels to animals, usually rodents, for the animals' lifetime. Such tests would be economically impractical for all chemicals to which man is exposed. New methods have recently been introduced to screen large numbers of chemicals quickly and cheaply which rely on the unifying hypothesis that all carcinogenic chemicals are electrophiles or must be converted to such by metabolism. These methods will be reviewed and compared with traditional methods of carcinogenicity testing, particularly as to their role in attempting to predict hazard to man.

Varying cancer incidence rates in different parts of the world, in different areas of countries and even between closely situated towns suggest that much human cancer may be largely determined by external factors. Individuals could respond in differing ways to these factors, thus accounting for the variation in cancer incidence within the same population. Similarly, studies of cancer incidence in immigrant populations show that individuals tend to lose the cancer site-spectrum and incidence characteristic of the country that they have migrated from and assume that of their new home. These facts suggest that cancer is to a large extent environmentally determined and therefore preventable (Doll 1967; Doll & Vodopija 1973; Saffiotti & Wagoner 1976; Doll 1977). This premise is increasingly being accepted for a number of reasons including: (1) cancer families are relatively rare; (2) there is little evidence of infectious transmission of human cancer; and (3) particular types of cancer can be explained by exposure to specific agents.

If one accepts the above statements, one can then go on to discuss how 'cancer' might be prevented. I wish in this paper to describe present methods of carcinogenicity testing which enable us to identify specific chemicals that can initiate cancer as well as methods that might be used in future screening programmes. It should be borne in mind at the outset that the rigour of any testing protocol will depend entirely on the exposure level to particular chemicals, on the duration of exposure and the number of people exposed to any particular substance(s).

Testing procedures for a food additive or pharmaceutical have in the past been more extensive than for industrial chemicals because of the greater numbers of

people exposed and the limited numbers of chemicals involved. This policy is now accepted as being socially unacceptable because it has meant that many industrial chemicals to which people were exposed had never been tested for toxicity. Nevertheless it is a sobering thought that if industrial chemicals were subjected to the same rigorous (perhaps too rigorous) toxicity tests as those required for pharmaceuticals, the cost in the United Kingdom alone would be some $£25 \times 10^9$ to test the backlog of chemicals and some $£250 \times 10^6$ p.a. thereafter. (These figures are based on a cost of £500 000 for a complete toxicity study; costs of carcinogenicity assays constitute approximately one-fifth of these figures.) Whatever one's views about the desirability or otherwise of conducting particular tests, it should be remembered that it is the consumer who pays, so that the money involved becomes an overriding factor. Profit margins are so small on some industrial chemicals that the cost of a pharmaceutical type toxicity test would not be commercially sensible and so the manufacture of the chemical would be discontinued with the consequent social effects. One cannot condone the manufacture of hazardous chemicals where there is human exposure but an attempt must be made to decide what is acceptable and what is not. Such decisions will have to be taken not only by scientists but by economists and others who are concerned with risk–benefit analysis. Clearly we cannot muddle along in the future in the same way that some companies do now or have done in the past in terms of determining the biological effects of chemicals. It is simply not acceptable to carry out a deliberate human toxicity study.

Animal carcinogenicity studies

What evidence is there to support the concept that cancer in man is in any way predictable? The strongest evidence must come from the finding that of those compounds or treatments that have been identified as causing human cancer, all with the possible exception of arsenic have been shown to be carcinogens in one animal species or another (see table 1). The question which is of prime importance is: are there any animal carcinogens which are not human carcinogens

Table 1. Human carcinogens active in animals

asbestos	bischloromethyl ether
ionizing radiation	benzene
ultraviolet light	mustard gas
2-naphthylamine	vinyl chloride
4-aminobiphenyl	stilboestrol
benzidine	aflatoxins
chrome ore	cyclophosphamide
nickel ore	synthetic steroids
polycyclic hydrocarbons	

and vice versa? It is on this point that there is a great deal of heated discussion at present over compounds such as amaranth, saccharin, cyclamate, phenobarbitone and so on. Of these compounds only the latter appears to have been tested

adequately (Ponomarkov *et al.* 1976; Rossi *et al.* 1977), data on the others have often been of a preliminary nature. In the final analysis, only epidemiological studies will tell us if there is any risk associated with these chemicals; no amount of animal experimentation can do this.

There have been many reviews, meetings and conferences on the extrapolation of animal carcinogenicity data to man. What emerges is that there are only a few compounds that have been adequately tested for carcinogenicity (see I.A.R.C. *Monographs on evaluation of carcinogenic risk of chemicals to man*). These animal studies must, however, provide the data base to validate all of the newer short-term tests for carcinogenicity that I shall discuss.

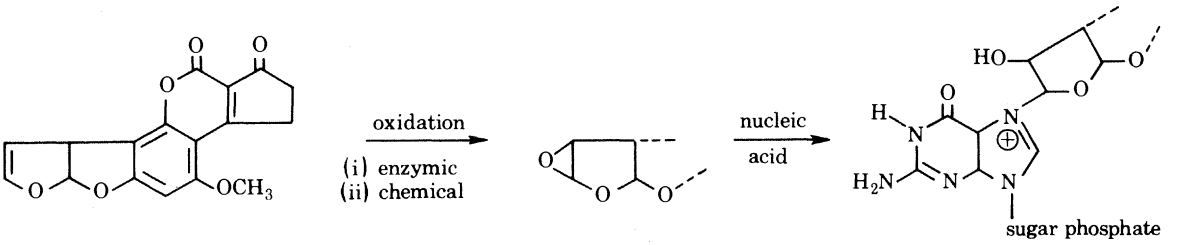
I do not wish to describe long-term animal studies in any great detail since I am not an expert on them. It is enough to say that the results obtained can be dependent on, for example, the animal species used (Clayson & Garner 1976), its sex, strain (Rueber 1976), nutritional status (Wattenberg *et al.* 1976), hormonal balance, purity of the compound tested, and numbers in the test and control groups (Fears *et al.* 1977). Despite this, the long-term feeding of chemicals to animals is our only well studied experimental method of determining the carcinogenicity or otherwise of a chemical. If one suspects that a particular compound is a carcinogen in humans the most definitive test to establish this must involve animal exposure. Such tests, as far as one can see, will detect all classes of agent known to be carcinogenic, i.e. physical, hormonal, viral and chemical, in contrast to some other of the tests which I shall describe.

Are there any unifying mechanisms of carcinogenesis?

Of the compounds listed in table 1 most are organic chemicals. I wish in the main to look at carcinogens in this class, primarily because their mechanism of tumour initiation is thought to be understood. That is, the initial interaction(s) of the organic chemical with biological material within the body is known. This is a long way from saying that we understand the mechanism of tumour production, but we can say at present what the first step is likely to be in the cancer process. This first step may also allow us to predict, on the basis of chemical structure, whether a compound is a carcinogen or not.

Administration of carcinogens to animals results often in the covalent binding of the chemical to macromolecules within the body. On the whole, the highest level of binding is found in organs susceptible to the particular carcinogen. These covalently bound adducts arise through metabolism of the carcinogen to a reactive chemical species (an electrophile) and subsequent reaction with nucleophilic sites contained within macromolecules. Figure 1 shows how aflatoxin B_1, a human carcinogen, is activated by liver mixed function oxidase enzymes to a reactive epoxide and also shows the structure of the major adduct found in nucleic acid consequent upon its reaction with guanine (Martin & Garner 1977). Carcinogenic susceptibility to aflatoxin B_1 could be dependent on how much epoxide metabolite is produced and how much reacts with critical target

macromolecules. This concept, of conversion of relatively inert chemicals to reactive species, has been proposed by several authors, particularly the Millers at the University of Wisconsin (Miller 1970), to be a common feature for most if not all chemical carcinogens (figure 2). Certainly the classes of chemical carcinogen which appear to fulfil this criteria are impressive. One can include the polycyclic



aflatoxin B_1

8,9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B_1

FIGURE 1. Activation of aflatoxin B_1 and subsequent reaction with nucleic acid.

aromatic hydrocarbons (Brookes & Lawley 1964; Sims & Grover 1974), various fused or conjugated aromatic amines (Clayson & Garner 1976), the nitrosamines (Magee *et al.* 1976), certain mycotoxins and natural products and a number of heterocyclic compounds. What is also impressive is that all of these classes react extensively with nucleic acids as well as proteins. Nucleic acid reaction is considered at present to be more important than protein reaction particularly because of the lack of experimental evidence to suggest that reaction with proteins can account for the heritability of cancer in daughter cells. A further reinforcement of the importance of nucleic acid reaction is the finding that the most potent animal carcinogen, aflatoxin B_1, is bound much more extensively to nucleic acid than to protein and that in a resistant species, the hamster, amounts of liver protein-bound carcinogen are similar to those in the rat, a susceptible species (Garner & Wright 1975). Naturally, gross differences in amounts of carcinogen binding can only give a crude estimate of the importance of reaction with any macromolecular type. What is also striking is the relation between chemicals that are carcinogenic in animals and those mutagenic in bacteria, indicating that nucleic acid reaction could well be a prerequisite for tumour production.

Electrophilic metabolite generation from carcinogens can therefore be presumed to be essential for cancer initiation for many organic chemicals. The events which determine whether an initiated or pre-neoplastic cell progresses to a frank malignancy are largely unknown. My discussion will therefore centre around what is known, namely that electrophilic metabolites initiate cancer, rather than the unknown events which determine whether a pre-neoplastic cell progresses to a fully malignant cell.

Methods of detecting electrophilic metabolites *in vitro*

Since electrophilic metabolites of carcinogens react readily with nucleophiles, measurement of such reactions gives a means of monitoring electrophilic metabolite production. Figure 2 shows various biological methods of detecting carcinogen

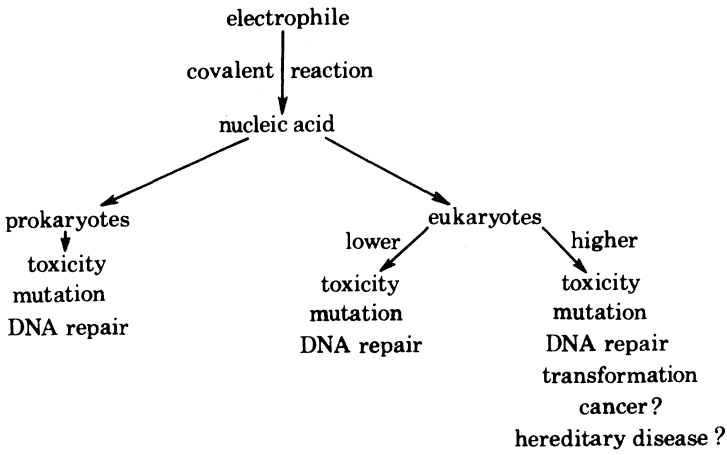


Figure 2. Consequence of electrophile reaction with nucleic acid in prokaryotes and eukaryotes.

reaction with nucleic acid in both prokaryotic and eukaryotic cells. The various techniques to be described have been reviewed by a number of authors (Magee 1974; Stoltz *et al.* 1974; Bridges 1976).

(*a*) *Nucleic acid reaction in prokaryotes*

Mutation as a result of exposure to an alkylating agent has been known for 30 years, but it is only in the last 6 years that the relation between mutagens and carcinogens has been proposed (McCann *et al.* 1975). This is because there was for a considerable time a failure to appreciate that carcinogens require metabolism to exert their effects and that most prokaryotes are unable to carry out these steps. Once it was accepted that oxidative metabolism was essential, this area expanded dramatically, so that at present there are few human or animal carcinogens which have not been shown to be mutagenic. It is perhaps natural that with this impressive correlation between mutagens and carcinogens there is now a tendency to assume that a particular substance is a hazard merely on the basis of its ability to induce mutations in bacteria. This is extremely unfortunate, because there is likely to be a strong reaction against this idea, the pendulum then swinging in the other direction so that it will be extremely difficult to convince people that any mutagen is a carcinogen.

Of the tests with prokaryotes in current use, mutation to prototrophy in *Salmonella typhimurium* is probably the most intensively investigated at the present time. The assay with the use of this microorganism involves co-incubation of a rat liver preparation with the carcinogen in the presence of the bacterial tester

strain, the ingredients being contained within a soft agar overlay (McCann *et al.* 1975) on a minimal agar plate or in liquid suspension (Garner *et al.* 1971; Malling 1971). The bacterial strains are all histidine auxotrophs, mutation being scored by counting the number of revertant colonies. A series of tester strains has been of theoretical objections can be made to this procedure but the fact is that at present constructed, allowing different classes of carcinogen to be detected. A large number an impressive correlation is found between compounds that induce mutation and their animal carcinogenicity. Of the *Salmonella* tester strains in use, I tend to view results obtained with strains TA1537 and TA98 with more caution than those obtained with TA1535 and TA100. This is because the latter two strains are base-substitution mutants. Reversion usually arises in these strains though covalent reaction of a carcinogen with nucleic acid, so disrupting normal base pairing or through error-prone repair pathways. The former two strains are both frame-shift mutants, and are reverted by intercalating agents such as the acridines. There is no evidence that these compounds are carcinogens until an alkylating moiety is attached to them. Since the hypothesis of metabolic activation states that most carcinogens are electrophiles, one has to be sure that the mutation observed is as a result of covalent DNA binding (Garner & Nutman 1977).

Other bacterial strains used for mutagenicity testing are tryptophan auxotrophs of *Escherichia coli* with varying DNA repair activity, and a multiple mutant strain of the same organism in which both forward and back mutation can be scored (Green & Muriel 1976; Mohn & Ellenberger 1973).

A simpler approach to detecting DNA damage in prokaryotes is to measure survival of isogenic bacteria with varying DNA repair capacities. Convenient tester strains are available for this type of assay, which depends on the fact that survival after exposure to DNA damaging agents will be dependent on the DNA repair capacity of the particular strain. The most sensitive tester strain to such agents would be one which is lacking both the *uvr* endonuclease and is also recombination deficient (Bridges *et al.* 1972). A comparison of the sensitivity of this strain with strains lacking only one of these repair pathways or the wild-type strain would show whether or not toxicity was due to DNA damage. Assays of this type are the so-called *rec-* or *pol*-assays (Kada *et al.* 1972; Slater *et al.* 1971). Neither of these have been extensively validated, although both appear to be suitable only for direct acting alkylating agents rather than those requiring metabolic activation. By using a liquid suspension assay and *E. coli* repair deficient strains, it was possible to show that the toxicity of an aflatoxin B_1 metabolite, the production of which was mediated by rat liver, was due to DNA damage rather than increased penetration of the activated metabolite (Garner & Wright 1973).

Finally, it is possible to measure DNA repair synthesis in bacteria as a result of carcinogen damage. This might have two advantages over mutagenicity assays in that only a single tester organism need be used and increased DNA repair could only arise through covalent reaction of the agent with nucleic acid (Thielmann 1976).

(*b*) *Nucleic acid reaction in eukaryotes*

Many of the assays of eukaryotes are basically the same as those that I have described for prokaryotes. What distinguishes these assays from bacterial assays is their increased complexity. Whereas a bacterial assay takes only 48 h to carry out from start to finish, a mammalian cell assay can take up to 6 months, depending on type.

There is now little doubt that mutations can be induced in mammalian cells in culture. Of the various techniques in current use, mutation to resistance to nucleic acid analogues appears to be the most widely preferred method. Resistance can be to compounds such as 8-azaguanine and 6-thioguanine (DeMars 1974; Shin 1974; Nikaido & Fox 1976). Recently, mutation to an alanine requirement has been demonstrated as well as reversion of the mutant cells by ultraviolet light, gamma rays and 4-nitro-quinoline-*N*-oxide (Suzuki & Okada 1976). For all of these mutation assays it is necessary to show that the mutant cells scored have a heritable mutation and that resistant cells do not arise through some other epigenetic mechanism.

Although only a few carcinogens have shown mutagenic activity in mammalian cells, this may be due to cells in culture having a low level of metabolizing enzyme activity. This problem can be partly overcome by the use of feeder cells but only if these have the necessary enzyme activity. To date, mutation in mammalian cells has only been studied extensively with the polycyclic aromatic hydrocarbon group of carcinogens (Huberman & Sachs 1974).

Survival studies with the use of human cell lines deficient in DNA repair have been suggested for carcinogen screening. At present, reports of this method have centred around compounds which act directly rather than requiring metabolism. The use of Xeroderma pigmentosum cells (an inherited human disease characterized by extreme sensitivity to ultraviolet light and a much increased skin cancer incidence) has shown these cells to be deficient in their ability to remove not only ultraviolet light damage (Cleaver 1970) but also chemical damage (Stich & San 1973). Agents which have greater toxicity in Xeroderma cells than in normal cells in culture are thought to be toxic by virtue of their reaction with DNA. The assay is similar therefore to the bacterial *rec-* and *pol*-assays previously described.

Reaction of an electrophile with nucleic acid in mammalian cells results in subsequent DNA repair. This can be monitored (1) by the non-S-phase uptake of [^{3}H]thymidine into nucleic acid measured by auto-radiography (San & Stich 1975) or scintillation counting of the extracted DNA (Martin *et al.* 1977), or (2) by looking for the presence of single strand breaks in the nucleic acid on alkaline sucrose density gradient centrifugation. A recent study has recently been completed at York on the testing of some 50 compounds of known carcinogenic activity for their ability to induce 'unscheduled DNA synthesis' in HeLa cells in culture. No known carcinogen was negative in this study with the exception of safrole. Four compounds, namely urethane, dimethylaminoazobenzene, and dimethylnitrosamine and diethylnitrosamine, which are weakly active or inactive

in standard bacterial mutagenicity assays, were active, and two compounds, 9-aminoacridine and sodium azide, which are mutagenic but not carcinogenic, were inactive. This assay would appear to be suitable for inclusion in any short-term testing protocol for carcinogens, providing a complementary assay to bacterial mutagenicity. HeLa cells have a low but perceptible capacity to activate carcinogens since all of those compounds which were active after liver metabolism had a low but significant effect in inducing 'unscheduled DNA synthesis' in the absence of liver. Increased sensitivity might be achieved by induction of the mixed function oxidase enzymes in these cells.

Finally in this section I shall discuss briefly transformation of cells in culture, that is the conversion of cells which display contact inhibition of growth to cells that have lost this property (Berwald & Sachs 1963; DiPaolo *et al.* 1971; Heidelberger 1973). Injection of the transformed fibroblasts into syngeneic animals should give rise to fibrosarcomas if the cells have genuinely been transformed whereas the original cell lines does not. Just what a transformed cell is, however, is not clear. Nearly all studies have used rodent cell lines which can be relatively easily transformed by chemicals; cultured human cell lines are more difficult if not impossible to transform by chemicals (Ponten 1976). Criteria for transformation are much discussed. Some people think that morphological criteria are sufficient to indicate transformation, others that the ability to grow in soft agar is a reasonable criterion for transformation whereas the purists think that transformation can only be demonstrated by malignant growth of transformed cells in animals.

Further assay procedures for transformation are the use of viruses in conjunction with chemicals. The virus itself does not transform the cell, and neither does the carcinogenic chemical, but the two in combination do (Freeman *et al.* 1973). Although this technique appears attractive, insufficient data are available at present to determine whether this type of test can be used routinely.

Other short-term tests for carcinogens

A great variety of other tests have been suggested to screen carcinogens quickly. Most of these have not been tested with large numbers of compounds. Although certain of them appear to be useful for particular classes of carcinogen, for example sebaceous gland suppression or non-specific esterase activity in sebaceous glands of mouse skin for the polycyclic hydrocarbon group of compounds (Healey *et al.* 1970), there is no evidence to suggest that they will become widely adopted in any routine testing protocol.

In-vivo tests of short duration for carcinogenicity

Many of the short-term tests described have a major disadvantage in that they use liver enzyme preparations, usually from a rat, to convert the test compound to a reactive metabolite. If activation of a particular compound goes through some mechanism other than mixed function oxidase attack or requires a further metabolic step, such as esterification, after oxidation, the requisite enzymes may not be contained within the liver preparation used. Furthermore, there is much evidence available to support the idea that tests *in vitro* give a measure of carcinogenic potential for a particular compound but not of species sensitivity or resistance. Thus a hamster liver preparation can activate aflatoxin B_1 to its 8,9-oxide to a much greater extent *in vitro* than can a preparation from the rat and yet this former species is resistant to the carcinogenic action of the compound whereas the rat is sensitive (Garner *et al.* 1972). In other words, there is an inverse correlation between carcinogenic susceptibility and the ability to activate this mycotoxin. Why this should be is not clear. It is possible that pharmacokinetic and pharmacodynamic parameters determine aflatoxin B_1 susceptibility and these cannot be mirrored in an artificial in-vitro assay. It is therefore essential, if we are interested in carcinogenic potency and hazard to man, to devise assays which can give us some idea of these two parameters.

What is clear at present is that there is no one animal species which we can say will behave in the same way as man. It has become popular to use non-human primates for this purpose but there is little if any evidence that this will be a useful approach. This brings us full circle therefore to in-vivo studies as the best method of attempting to predict hazard to man. What techniques are available, to speed up the process of carcinogenicity testing in animals, that do not involve the vast expense of traditional methods? Approaches to this problem which have been suggested are the use of strain A mice and the scoring of lung adenomas (Shimkin *et al.* 1966). This approach, while quicker than conventional studies, may be unsatisfactory because of variable spontaneous tumour incidence and the great number of factors which can alter this incidence in the control population.

Another approach is that of scoring the numbers of transformed cells taken from hamster foetuses after administration of the carcinogen to the mother. This assay, which is an in-vivo/in-vitro assay has the advantage of using whole animals but may be subject to problems associated with the species used (DiPaolo *et al.* 1972). Should one use hamsters or rats or mice and what criterion of transformation should be used? A further approach is to treat the whole animal with the test compound and then remove and culture particular organs. In this way the conversion of a pre-neoplastic cell to a frank malignancy can be speeded up (Mondal 1975). This approach seems promising on paper but, like all the tests I have described, looks only at the initiation stage of carcinogenesis rather than other stages which are said to play an important role in tumour progression.

If methods could be devised to pick up pre-neoplastic changes early on, such

as the production of embryonic antigens, then this type of test would obviously be of great advantage in the screening of compounds for potential carcinogenicity. Finally, there are methods that show that DNA damage has occurred *in vivo*; these methods employ density gradient centrifugation techniques of DNA from target organs but would appear at present to be too tedious for the routine screening of carcinogens (Cox *et al.* 1973). A procedure which may have some use in detecting the covalent reaction of carcinogen with DNA would be the monitoring of excreted carcinogen adducts in the urine of carcinogen treated animals, possibly by radioimmunoassay. This could be a sensitive technique provided the adducts were excreted unchanged and that the necessary antibodies could be raised.

Determination of priorities after carcinogen testing

Various schemes have been proposed to determine which assay should be carried out in any testing protocol so that priorities for long-term carcinogenicity tests can be determined (Bridges 1973; Flamm 1974; Bartsch 1976). Since carcinogens react with macromolecules, tests that depend on this for their function should be used. It is obvious from the foregoing sections that schemes should start off with the simple and move to the more complex. Thus mutation in microorganisms should be the initial assay system for routine screening of chemicals. Though the techniques are relatively simple, the results can often be complex. One knows the constituents of the assay and the end-point; however, the great number of factors which might be important in reaching the end-point of mutation should not be forgotten. These can include the role of bacterial metabolism in activation and detoxification of metabolites, aerobic oxidation of metabolites, the close proximity of bacteria and endoplasmic reticulum, the possibility of reactions of metabolites with the parent compound or other metabolites and the concentration of substrate and cofactors. Furthermore, it should be recognized that there are some chemicals which are active mutagens only if the assay is carried out in a particular manner. Thus, dimethylnitrosamine and diethylnitrosamine are only weakly or non-mutagenic in a standard plate test but active in a liquid suspension assay.

Accepting that bacterial mutagenicity is the simplest screening procedure we can use routinely, what other tests need to be done in conjunction with this assay to increase the reliability of predicting whether a particular chemical has carcinogenic potential? Studies in our laboratory indicate that as previously mentioned, the measurement of 'unscheduled DNA synthesis' in HeLa cells in culture with the addition of a liver enzyme preparation gives a reliable indication that a particular chemical is activated to an electrophilic species (Martin *et al.* 1978). It is my opinion that these two tests together give good predictive value for carcinogenic potential and can be used for pre-screening.

Compounds giving positive results in both tests should be viewed with suspicion. If the positive compound is part of a structural series, other members of which are negative and these have the desired activity, then naturally one should concentrate one's attention on the non-mutagenic compounds. If there is no

alternative chemical or the benefits far outweigh the hazard, the necessary handling precautions should be taken to avoid heavy exposure of people employed in its manufacture. There are certain compounds for which, on the basis of their chemical structure, one can predict with some accuracy whether the compound might have carcinogenic potential. It has been suggested that in a short-term assay a structurally related compound of known carcinogenicity should be tested in conjunction with the test compound as a positive control. Naturally if the assay is negative for the known carcinogen it is unlikely to pick up activity from the test compound (Purchase *et al.* 1976).

Some compounds which are hormonal in their action, or which act through some physical characteristic such as fibre size, would have to be tested in animal studies immediately, without the necessity of doing a short-term test since most of these would not pick the compound up anyway. Chemicals such as diethylstilboestrol might be active in a short-term test but their action may not be related to their mechanism of initiating cancer. It is therefore pointless to try to find one or more assays that will detect every type of carcinogenic substance no matter what its mechanism of action.

Conclusions

The short-term tests that have been described here enable one to make a rational approach to the testing of chemicals for tumour initiating activity. They cannot, at the present time give any quantitative idea of hazard to man nor of carcinogenic potency (long-term animal studies may not be able to do this either). The tests can also provide a method of monitoring people for exposure to carcinogens by assaying urine extracts for mutagenicity. The use of the tests is in a toxicological testing programme for all compounds to which people are exposed. If closed processes are involved in manufacture, it is pointless testing these compounds unless individuals come into contact with them.

Finally it should be pointed out that there are many arguments over the predictive value of animal carcinogenicity tests. Most short-term test programmes have, however, depended heavily on the results of these to establish their predictive value. One hopes that this is not a question of two imponderables being added one to another.

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Discussion

G. A. H. Elton (*Ministry of Agriculture, Fisheries and Food, London SW1P 2AE, U.K.*). Environmental contaminants can affect man by various routes, one of which is via food. For example, for the average person, food is the main source of intake of lead and of mercury, and virtually the only source of intake of methyl mercury. Food additives and their reaction products with food components are also ingested.

In attempting to assess the risk, if any, to the population from additives and contaminants in food, we sometimes have assistance from guide-lines from W.H.O. and other medical authorities (e.g., for lead and for methyl mercury); these guide-lines are usually based on information about the toxicity for man of the material under consideration, taking into account industrial exposure, accidents, etc. For many substances, however, such information does not exist or is at best fragmentary, and we have to rely on information based on animal experiments and/or in-vitro studies. The interpretation of such information poses many problems, particularly in relation to possible carcinogenicity. Cancer risks can only be assessed in terms of probabilities; for example, at best one can only hope to be able to say that the 'average man (or woman)' exposed to compound A at x % in the diet for y years has a probability z of incurring cancer as a result. How can one make such an assessment for a population of many millions of people, eating varied diets, particularly when both x and z are very small? Studies on a few hundred animals are of limited use. To obtain positive results with a reasonable number of rats, for example, many research workers feed the test compound at levels up to the highest dose that the rat can tolerate (sometimes 5 % by mass of the diet or more). Extrapolation of the results for high dose levels to more realistic levels of ingestion (perhaps a few parts/10^6 or parts/10^9) is hazardous, especially when one is attempting to extrapolate almost to the origin of the dose–response graph. It is then still necessary to take the additional step of extrapolation from rat or other experimental animal to man.

These are fundamental problems of risk assessment. How far we can contribute to their solution by the use of short-term tests based on microorganisms or on mammalian cells remains to be seen. Meanwhile, risk assessment for additives and contaminants in food will continue to be based on skilled judgement by experts of data which are not always complete, but on which decisions must be taken to protect the public safety. Furthermore, we must always be prepared to revise our judgements at short notice if necessary, as valid new experimental evidence becomes available.